Aus der Medizinischen Klinik der Universität Bern
Direktor: Prof. Dr. W. Hadorn

Institut für Physikalische Therapie
Arbeit unter der Leitung von Prof. Dr. med. M. Gukelberger

SEROLOGICAL DETERMINATIONS OF RHEUMATIC ACTIVITY

INAUGURAL-DISSERTATION

zur Erlangung
der Doktorwürde
der Medizinischen Fakultät
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vorgelegt von

Robert Dunkelman

von Pompton Plains, New Jersey, U.S.A.

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Purpose

Up to the present time, the generally accepted procedure in following the course of rheumatic activity has been by means of the erythrocyte sedimentation rate (ESR). This procedure has proven itself extremely useful, but the influence of several factors have limited its value and have created the need for a more unequivocal test. These factors will be discussed in greater detail later. It has been the purpose of this work to attempt to compare two other laboratory procedures with the ESR and to show the advantages and disadvantages of all the three methods involved. These two procedures, the diphenylamine (DPA) reaction and the C-reactive protein (CRP) precipitin test, have primarily been used to demonstrate the degree of rheumatic activity in the sera of patients with rheumatoid arthritis, both acute and chronic. Sera of patients suffering from other diseases, both related and non-related, have been also tested in an attempt to correlate the specificity of the reactions. A careful control of normal sera has been carried out along with the testing of pathological sera in order to establish a norm.

Procedures

THE DIPHENYLAMINE (DPA) REACTION

The serum diphenylamine reaction, a "semimicro" procedure, used by Coburn and Haninger (1), and first described by Ayala, Moore and Hess (2), was used with slight modifications in this study. Sugar ratios were determined by the method described by Sonntag (3).

The tissue injury which occurs with an inflammatory reaction may be reflected in the blood in several ways (1). Kelly and his associates have shown that there is a correlation between the serum mucoprotein level and the erythrocyte sedimentation rate (4). Siebert et al. have presented considerable evidence that an increase in polysaccharide, as well es in alpha$_2$ globulin, indicates the presence of tissue destruction (5). They pointed out a possible correlation between these levels and the erythrocyte sedimentation rate.

The serum DPA reaction test is a colorimetric determination which is not specific for any one type of joint disease, but which gives a clear indication as to the activity of the disease as reflected through tissue injury. The method may be used to determine the activity of any type of joint manifestation regardless of its etiology (3). The DPA rise appears to be associated with the tissue injury that follows

joint swelling. The degree of the DPA rise is determined by the severity of the inflammatory process, and the amount of anti-inflammatory agent required to suppress a rise depends on the intensity of the reaction (1).

The serum constituent which reacts with DPA to give a purple color is unknown. It seems to be constantly associated with mucoprotein, not only in serum, but also in urine, gastric fluid, synovial fluid, tonsillar tissue and collagen. The reactive material appears to be a carbohydrate in nature. In screening carbohydrates, it has been found that several types of uncommon sugars give this reaction (1).

DETERMINATION OF THE SERUM DIPHENYLAMINE REACTION

Two reagents must be prepared. The first is a mixture of 90 ml. of glacial acetic acid and 10 ml. of concentrated sulfuric acid with 1 Gm. of DPA (recrystallized from ethanol) dissolved in it. This will be referred to in this study as r e a g e n t w i t h D P A. The second reagent is the same proportions of the acids mentioned above, but without DPA. This will be referred to as r e a g e n t w i t h o u t D P A.

5 ml. of a 5% trichloracetic acid solution is added slowly, with gentle shaking, to a 0.2 ml. sample of serum. The test tube is then stoppered with a rubber stopper which has been fitted with a 20 cm. long glass tube to act as a condenser, and placed in a boiling water bath for exactly 20 minutes. After 20 minutes, the tube is immediately cooled by immersion in ice water. Following the cooling, the hydrolysate is filtered and 1.5 ml. of the clear filtrate is transferred to each of two fresh tubes respectively.

To the first of these tubes is added 3 ml. of reagent with DPA. This will give the desired purple color. To the second of these tubes is added 3 ml. of reagent without DPA. This mixture will give a brownish color, the optical density of which must be subtracted from that of the purple color reaction in the first tube. This brownish color is caused by the action of sulfuric acid, and varies with each serum sample. It is, therefore, necessary to prepare this blank and determine its optical density separately for each individual serum sample in order to obtain a corrected optical density for the purple color DPA reaction.

A second blank consisting of 1.5 ml. of 5% trichloracetic acid and 3 ml. of reagent with DPA but no serum, is also prepared. This blank is used to establish the zero optical density setting on the spectrophotometer.

All these tubes, including the blanks, are immersed in a boiling water bath for exactly 30 minutes after which they are cooled in ice water and allowed to rise to room temperature. The color intensities which develop are stable at room temperature for several hours.

In this study, an additional control, described by Sonntag (3), was used as a check and also to arrive at a final set of results which could easily be duplicated on various types of spectrophotometers. This consisted of 0.2 ml. of a 1% glucose solution which was treated exactly as the 0.2 ml. serum sample. It was hydrolysed with 5 ml. of 5% trichloracetic acid, and 1.5 ml. of the hydrolysate was treated with 3 ml. of reagent with DPA. A light blue color resulted, the optical density of which was also determined (3).

Gross changes in the optical densities of these sugar control solutions, which should remain fairly constant, would show either changes in the reagents used or changes caused by heating too long or too short a time. The practice of running such a sugar control simultaneously with each serum sample, or group of samples, not only provides a close check on the reliability of the results, but also makes possible the determination of a Sugar Ratio (SR) for each serum sample. This SR, which is the result of dividing the corrected optical density of the DPA purple color by the optical density of the sugar control, gives a numerical result of far greater significance than a mere instrumental reading, for it can be reproduced on any type of instrument (3).

The spectrophotometer used in this study to determine optical densities was a "Photometer Eppendorf" manufactured by Netheler and Hinz GMBH, Hamburg, Germany. Yellow filters 578 and measuring chambers 1 cm. thick were used for all determinations. The readings were from the logarithmic scale of the instrument.

It was the practice, during this experimentation, to prepare fresh solutions of reagents each week. All serum specimens were kept under refrigeration and no specimen was more than three days old. Any specimen in which hemolysis took place was discarded.

THE C-REACTIVE PROTEIN (CRP) PRECIPITIN TEST

Tillet and Francis (8) have shown that by various inflammatory processes an abnormal protein appears in human serum, which can be demonstrated during the acute phase of the infectious disease. They have shown that in the presence of somatic C-polysaccharide of pneumococcus a precipitin reaction appears and thus the name "C-reactive protein". Also because of the time of its appearance in blood, it can also be called the "acute phase protein". It gave no reaction with the sera of the same patients during convalescence. This reaction was found to be non-specific for pneumococcal infections but was also noted in the sera of patients with acute rheumatic fever, staphylococcal osteomyelitis and subacute bacterial endocarditis (8). Several chemical and immunological properties of this substance were observed through a series of experiments. It wanders with the a-globulinfraction

and is destroyed by heating to 65° C. Several properties show it to differ from true antibodies (9, 12, 13).

Mc Leod and Avery (9) were the first to isolate the CRP and prepare it in its pure form. They produced a CRP-antiserum by immunizing rabbits with the pure CRP. This CRP-antiserum reacts with traces of CRP by means of a precipitin reaction. The CRP-antiserum does not react with the proteins of normal human serum. The sensitivity of the tests for CRP, using the antiserum, was greater than that using the C-polysaccharide so that smaller amounts could be detected (9). The simple method of determination of the CRP by means of the CRP-antiserum is applicable as a routine procedure and permits a semi-quantitative determination (7).

DETERMINATION OF THE C-REACTIVE PROTEIN REACTION

The method for determination of the CRP is the precipitin reaction described by Anderson and Mc Carty (7). Capillary tubes with an internal diameter of 0.4 mm and length of 90 mm are used. A capillary tube is filled 1/3 with CRP-antiserum and then with the same amount of native serum from the patient. A separation of both sera by an air bubble is to be avoided. By turning, the fluid is brought to the center of the capillary tube which is then placed in a capillary stand. The capillary is then incubated for two hours at 37° C. After about thirty minutes a precipitate will appear if CRP is present in the patient's serum. After two hours of incubation, the reaction can already be observed and judged but the final reading is taken after the capillary has been left under refrigeration at 4° C over night. The reaction is read with + to ++++ according to the amount of precipitate present (7). If no mentionable amount of CRP is present in the serum, no visible reaction will appear. Minute amounts of CRP (less than 0.01 mg/ccm) can be determined by this method (10).

A specific positive control for the CRP-antiserum test can be run off simultaneously. This is the CRP-Positest, which is a standardized "acute phase serum" preparation used as a control for confirming technique as well es the CRP-antiserum's accuracy. This control is performed exactly as the CRP-antiserum test, with the exception that CRP-Positest is substituted for the patient's serum. Within twenty minutes, a positive reaction will appear. After incubation for two hours, the reaction will reach a +++ magnitude, and this may be kept as a reference. The CRP-Positest eliminates doubt in borderline cases by confirming negative reactions and guards against errors in technique.

The CRP-antiserum and CRP-Positest are obtainable from Schieffelin and Co., 18 Cooper Square, New York 3, N.Y.

Experimental findings

It was necessary to establish a control so that a norm could be determined as a basis for comparison. Ten healthy individuals were chosen, of which five were male and five female. None had any previous history of rheumatic disease. The highest control had an SR of 4.509 and the lowest an SR of 3.400. An average SR of 3.941 was found for the entire group. The CRP was negative in all cases.

NORMAL CONTROLS

	SR (DPA)	CRP
Control No. 1	3.400	neg.
Control No. 2	3.491	neg.
Control No. 3	4.182	neg.
Control No. 4	4.509	neg.
Control No. 5	4.333	neg.
Control No. 6	4.205	neg.
Control No. 7	3.774	neg.
Control No. 8	3.520	neg.
Control No. 9	4.020	neg.
Control No. 10	3.980	neg.
Control Average	3.941	neg.

Five cases of acute rheumatoid arthritis were tested. The diagnoses were established on the basis of clinical findings. In all cases the SR was above the normal value and the CRP was positive. Two of the cases showed a normal ESR.

ACUTE RHEUMATOID ARTHRITIS

	Sex	Age	SR (DPA)	CRP	ESR (1st hour)
Case No. 1	F	52	6.442	++	47
Case No. 2	M	38	6.762	+++	47
Case No. 3	M	48	4.963	+++	36
Case No. 4	F	62	7.884	++	10
Case No. 5	F	26	5.955	+	4

Six cases of primary chronic polyarthritis in acute exacerbation, which were clinically diagnosed, were tested. In all cases the SR was elevated and the CRP was positive. The ESR was significant in all the cases. While the males outnumbered the females in this group, it was noted that the youngest patient was 53 years old.

PRIMARY CHRONIC POLYARTHRITIS IN ACUTE EXACERBATION

	Sex	Age	SR (DPA)	CRP	ESR (1st hour)
Case No. 6	M	56	5.874	+	35
Case No. 7	F	77	8.745	++	65
Case No. 8	M	62	7.804	++	100
Case No. 9	M	55	5.527	+	42
Case No. 10	F	55	5.127	+	20
Case No. 11	M	53	8.961	++++	55

There were 22 patients in the group with primary chronic polyarthritis. Of these an equal number were male and female. The ages ranged from 32 to 78 years of age. Two of the patients showed a normal SR while the CRP was negative in all cases. There was no significance regarding the ESR as 15 of the patients were within normal limits.

PRIMARY CHRONIC POLYARTHRITIS

	Sex	Age	SR (DPA)	CRP	ESR (1st hour)
Case No. 12	M	32	6.477	neg.	5
Case No. 13	F	34	7.000	neg.	12
Case No. 14	M	78	11.712	neg.	45
Case No. 15	F	43	6.328	neg.	12
Case No. 16	F	46	6.354	neg.	11
Case No. 17	M	63	10.548	neg.	21
Case No. 18	F	72	5.509	neg.	8
Case No. 19	M	53	6.961	neg.	10
Case No. 20	M	46	6.314	neg.	3
Case No. 21	F	74	8.177	neg.	45
Case No. 22	F	53	6.382	neg.	18
Case No. 23	F	48	7.720	neg.	12
Case No. 24	M	67	7.364	neg.	9
Case No. 25	M	70	5.865	neg.	16
Case No. 26	F	69	8.011	neg.	28
Case No. 27	M	44	4.206	neg.	6
Case No. 28	F	52	5.302	neg.	5
Case No. 29	M	58	5.217	neg.	3
Case No. 30	M	58	6.677	neg.	12
Case No. 31	F	37	4.302	neg.	3
Case No. 32	F	39	5.008	neg.	4
Case No. 33	M	61	6.709	neg.	11

Special interest was accorded the group of patients with degenerative joint disease, in order to determine whether the serological methods could be used as a possible means for the differential diagnosis from rheumatoid arthritis. Degenerative joint disease, also known as osteoarthritis or arthrosis deformans, has shown a SR increase in all cases while the ESR remained normal. Five serum samples were tested and the CRP was negative in all cases. As was to be expected, all the patients were 45 years of age or older. The increased SR seemed to be correlated with the number of joints involved in each particular case.

DEGENERATIVE JOINT DISEASE

	Sex	Age	SR (DPA)	CRP	ESR (1st hour)
Case No. 34	F	45	7.636	neg.	11
Case No. 35	F	54	6.318	neg.	12
Case No. 36	F	60	6.318	neg.	8
Case No. 37	M	45	5.500	neg.	2
Case No. 38	M	67	7.208	neg.	6

Serum samples of patients with diseases entirely non-related to the arthritic group were also tested. This was done to see if the DPA reaction and the CRP-antiserum test were specific to any one entity. It was observed that a positive CRP can be produced by several diseases of an infectious nature. The DPA rose in all cases, proving that tissue destruction was in progress. It was also noted that in cases where the CRP was positive, the ESR was also increased. The DPA rise could not be correlated to this. There was also no correlation between sex and age to the disease process.

NON-RELATED DISEASES

	Sex	Age	SR (DPA)	CRP	ESR (1st hour)	Disease
Case No. 39	M	63	9.442	+	92	Pyelonephritis
Case No. 40	F	58	5.545	neg.	8	Diabetes mellitus
Case No. 41	F	50	5.109	neg.	8	Pyelitis
Case No. 42	F	35	5.582	++	20	Cholecystitis

Two studies were carried out on synovial fluid instead of serum. Both cases were primary chronic polyarthritis in acute exacerbation. Although a DPA rise was noted, the CRP remained negative. Case No. 43 and Case No. 8 in this thesis are actually the same patient. In the former synovial fluid was tested, where as in the latter serum was tested. It is noteworthy that the CRP was of a ++ magnitude in serum but negative in synovial fluid. The SR was also higher in the serum than

it was in the synovial fluid. While this is of interest, no conclusions can be drawn because it is the only case that was doubly tested. It seems to suggest however, that while the CRP may present in serum, it is probably absent in synovial fluid.

PRIMARY CHRONIC POLYARTHRITIS IN ACUTE EXACERBATION
(SYNOVIAL FLUID)

	Sex	Age	SR (DPA)	CRP	ESR (1st hour)
Case No. 43	M	62	6.750	neg.	100
Case No. 44	M	27	5.145	neg.	8

It may be interesting to note that of the 44 patients tested, 22 were male and 22 female. Their ages ranged from 26 to 78 years of age. The youngest male was 27 and the oldest 78. The youngest female was 26 and the oldest 77.

Discussion and Correlations

The results of this study clearly demonstrate that the DPA reaction is not in any way specific for any particular type of joint involvement. It cannot even be said that a rise in DPA is diagnostic for joint disease in general, because it was clearly shown that tissue destruction or injury regardless of place or origin will cause a rise. While the DPA reaction definitely indicates that tissue destruction is in progress, it can be used as an aid only after the clinical diagnosis has definitely been established. In other words, once the clinician has established his diagnosis, the serum DPA reaction can be used to aid him in determining how far the disease process has progressed.

The administration of sodium salicylate, cortisone, hydrocortisone and splenin A prevented any rise in DPA values. It has been observed that although the DPA values may decrease under corisone therapy, these values will remain significantly high as long as the disease continues to produce objective and subjective symptoms (1). The serum DPA reaction may be of definite value in determining the progress of the patient under therapy. In this way therapy may be increased or decreased.

There was no definite correlation between SR values and the ESR in this study. While both of these values tended to be high in acute processes, the SR remained high and the ESR was lower in the chronic cases.

This study has shown that the serum DPA reaction may be of definite value in the testing sera of patients with degenerative joint disease. This disease is characterized by the destruction of tissue without any infectious involvement. The degree of tissue injury may be determined and thus give the physician an overall picture of the disease progress.

An advantageous feature of the serum DPA reaction is that it can be carried out in any laboratory with a minimum of training for the technician. All reagents are readily available. It does, however, involve a considerable amount of time and it is suggested that a series of serum samples be run off simultaneously.

It was also noted that the rise in DPA values seemed to be in accord with the number of joints involved. It must therefore be kept in mind that a high rise must be correlated with the number of joints involved. When a high serum DPA rise occurs in the presence of singular joint involvement it must be evaluated differently than when the same value is reached in the presence of multiple joint involvement. In other words, the same amount of tissue damage is possible in one joint as in many joints. Estimation of total tissue injury and correlation of the serum DPA rise is a matter of experience on the part of the clinician as a definite scale of values regarding the number of joints involved remains to be determined.

Before discussing and evaluating the CRP reaction, it is necessary to discuss the ESR and the need for its replacement by another test. It has previously been mentioned that although the ESR has been extremely useful in determining the presence of an inflammation, a number of factors influence the fall of the red blood corpuscles. The ESR is increased in many anemias because the number of red blood corpuscles is reduced. It is also commonly acknowledged that one of the secondary symptoms of rheumatoid arthritis is anemia. Therefore, any anemia, whether primary or secondary in nature, is bound to have a marked effect on the ESR and its evaluation.

The plasma fibrinogen is often increased during pregnancy and an ESR evaluation is not a reliable indication of inflammation. The fibrinogen increase will elevate the ESR. In some acute infections, such as lobar pneumonia, the fibrinogen is also increased and the ESR will remain elevated long after the acute process has subsided, because the return of the fibrinogen to normal levels is often very slow. Fibrinogen is decreased, on the other hand, in congestive heart failure, due to passive liver congestion. The ESR is frequently normal in such patients irrespective of whether or not an inflammatory process is present.

In individuals with hyper globulinemia, idiopathic or due to multiple myeloma, and in individuals with low serum albumin due to nephrosis the ESR is elevated. In such cases the ESR is unreliable as an indicator of inflammation.

As previously pointed out, the CRP precipitin test depends upon the appearance in human serum, during acute inflammations, of an abnormal protein known as the CRP (8). In acute streptococcus infections such as angina and pharyngitis, which are considered as forerunners of acute polyarthritis, a CRP reaction can be shown usually at the moment of onset of clinical manifestations, even before an ESR increase can be observed. If the process subsides quickly the CRP returns rapidly towards negative, even though the ESR is at its maximum or still relatively high. Parallel to the decrease of the CRP the normalization of the white blood cells can be observed in most cases (6). Later complications in the form of rheumatic disease are characterized by a prompt increase of the CRP, which in the interval between the angina and polyarthritis remain in lesser amounts in the serum (6, 10) or during this time disappear completely (11).

The results of the study on the occurrence of CRP in patients with acute rheumatic fever (7) suggest that its presence in the blood is a sensitive, and perhaps the most sensitive indication of activity of the disease process. It is the most consistently positive laboratory test in the presence of rheumatic activity. The changes in the amount of CRP present tend roughly to parallel the changes in the ESR but this is by no means invariably true since CRP may be absent from the blood when the ESR is much higher than normal. In fact, in the early recovery period of rheumatic fever the CRP usually becomes negative before the ESR has returned to normal. This is not an invariable relationship, however, and the CRP may be present in blood when the ESR is normal. This discrepancy probably indicates that CRP is not in any important way related to or responsible for changes in sedimentation velocity although the point has not been conclusively tested experimentally (7).

Experimentation by various diseases have shown that the presence of CRP is not specific for definite disease groups, as it is present in many inflammatory processes of infectious and non-infectious origin (7, 8). For this reason, its presence in serum cannot be considered for diagnostic values, but similarly to the ESR, gives a sensitive index of the present disease activity. It is, however, an extraordinary sensitive indicator for inflammatory processes and its presence has proven itself very valuable for the determination of the activity of certain diseases, especially those of rheumatic nature (6).

The decrease of CRP in serum was noted while the ESR still remained high during the period of convalescence or under therapy. When the rheumatic process became inactive the CRP decreased rapidly while the ESR remained increased for another 2 to 5 weeks. Anti-rheumatic therapy causes the CRP to decrease in most cases. Hormonal preparations such as ACTH, cortisone and Decortin are more effective than Butazolidine, Irgapyrine and salicylic acid. Therapy is said to be effective when it can cause the CRP to disappear from the blood within 7 to 10 days. If the

CRP does not respond to therapy, it does not necessarily mean that the medication is not effective but that the dosis is not sufficient. If the medication did not cause a decrease in the CRP, a change in medication often did (6).

The results are recorded on the basis of the volume of the precipitate formed, ranging from zero in the absence of reaction to ++++ in the case of a maximal reaction. It is obvious that readings of this sort may not be the same when made by different observers, and furthermore different lots of anti-serum vary in the amount of precipitable antibody which they contain (10). The CRP precipitin reaction can easily be used as a general procedure. It has the advantage of requiring only very small quantities of serum and antiserum. One drawback is the difficulty in obtaining antiserum (7). The procedure, itself, is relatively simple and can be carried out with ease by any technician. The final readings should be made by an individual who has had practice and experience.

The results of this study have definitely shown the value of the CRP precipitin test as an indicator of rheumatic activity. Positive results were obtained only in acute cases or chronic cases in acute exacerbation. Negative results were seen in all chronic cases. There seemed to be a correlation between a positive CRP reaction and an ESR increase in most cases although the degree of this was inconsistent. Positive results were seen in some non-related cases with inflammatory processes, indicating that the CRP reaction is non-specific.

There was no correlation between the CRP reaction and a DPA rise as both of these tests depend upon entirely different factors. A highly positive CRP result can show a small DPA rise because there is no necessary relationship between inflammation and tissue injury. The converse of this was also shown to be true.

A single common factor between the serum DPA reaction and the CRP precipitin test is that under anti-rheumatic therapy both will tend to return to their normal levels.

Like the DPA reaction, the CRP precipitin test is most useful when a clinical diagnosis has been previously made. This is, of course, due to the fact that the CRP reaction is non-specific. Once the clinician has made the diagnosis of rheumatic activity of an inflammatory nature, the CRP will give him a definite evaluation as to its degree. It will also offer valuable aid in appraisal of the therapy given.

Conclusions

The serum DPA reaction is a non-specific method for the determination of tissue injury regardless of etiology. It cannot be used as a diagnostic procedure but is

of definite value in evaluating the progress of a disease process. A serum DPA rise is observed in rheumatic activity but its greatest value is in estimating the degree of tissue destruction in degenerative joint disease.

This study has found no definite correlation between DPA values and the ESR. While both of these values tended to be high in acute processes, the DPA value remained high and the ESR was lower in the chronic cases. There was no correlation of the DPA values with age and sex.

It has been noted that the serum DPA tends to rise with the number of joints involved. This is most markedly observed in degenerative joint disease with multiple joint involvement.

Perhaps the most important feature of the serum DPA determination, other than estimating the degree of tissue injury, is the evaluation of concurrent therapy. As medication is administered, the serum DPA will decrease. Once the clinician has established his diagnosis, the serum DPA reaction will thus afford him valuable assistance.

The CRP precipitin test is also a non-specific test, but for the evaluation of the degree of an inflammatory process. It has been compared to the ESR in this study. The ESR has a number of factors which influence its determinations and so it has been one of the purposes of this study to see if it can be replaced by the CRP precipitin test.

While the CRP test has been found to show positive results in many inflammatory processes, it has proven itself to be an excellent indicator for acute rheumatic activity. According to Anderson and Mc Carty (7) the CRP determination is considered to be probably the finest indicator for the activity of rheumatic processes that is known today. Its greatest value lies in evaluating the inflammatory degree of acute forms and it is of no value in chronic forms.

The CRP precipitin test is an excellent indicator in the progress and effectiveness of therapy. It was more reliable than the ESR, which often continued to remain high long after the process became inactive (6).

Like the serum DPA reaction, the CRP precipitin test is most useful after a clinical diagnosis has been established. This is, of course, in regard to assisting the clinician to evaluate the degree of inflammation and effectiveness of the therapy.

When carried out simultaneously, the serum DPA reaction and CRP precipitin test can be of definite assistance to the clinician.

Zusammenfassung

Die vorliegenden Untersuchungen befassen sich mit der Frage, inwieweit der Nachweis des C-reaktiven Proteins sowie die Diphenylaminreaktion des Serums zur Beurteilung der Aktivität eines entzündlich rheumatischen Prozesses herangezogen werden können. Bei beiden Proben handelt es sich um unspezifische Reaktionen.

Die Diphenylaminreaktion beruht nach Coburn und Haninger auf dem Nachweis eines im Serum vorhandenen Mucoproteins, dessen chemische Konstitution noch nicht bekannt ist. Es kommt unter anderem im kollagenen Bindegewebe vor und soll bei jedem Bindegewebsabbau in vermehrtem Masse ins Blut übertreten, wobei die Art des Bindegewebsabbaus (entzündlich, degenerativ) offenbar von untergeordneter Bedeutung ist (Sonntag).

Beim Nachweis des C-reaktiven Proteins (CRP-Reaktion) handelt es sich um eine Precipitinreaktion des Serums mit dem C-Polysaccharid der Pneumococcen. Diese Reaktion fällt bei den verschiedensten akuten Infektionskrankheiten positiv aus, steigt im allgemeinen rascher an als die Senkung und soll schon nach Abklingen der akuten Phase wieder negativ werden. Beim akuten Gelenkrheumatismus ist sie angeblich sehr stark positiv, beim primär chronischen Gelenkrheumatismus dagegen stets negativ. Da jedoch im Verlauf der primär chronischen Polyarthritis auch akute Schübe vorkommen, blieb abzuklären, ob die CRP-Reaktion auch in dieser Phase negativ bleibt.

Für die Durchführung der Diphenylaminreaktion bedienten wir uns des Verfahrens von Coburn und Haninger, modifiziert nach Sonntag, während die Bestimmung des C-reaktiven Proteins mit dem Gerät der Firma Schieffelin, New York, und dem damit gelieferten CRP-Antiserum erfolgte. Die Resultate beider Teste wurden schliesslich mit der Senkungsreaktion verglichen. Dabei ergaben sich folgende Resultate:

1. Unter normalen Bedingungen:

   Bei 10 gesunden Versuchspersonen mit normalen Senkungswerten von 2 - 8 mm fiel der Nachweis des C-reaktiven Proteins stets negativ aus. Die dazugehörigen normalen Werte für die Diphenylaminreaktion schwankten zwischen 3,4 - 4,5 bei einem Mittelwert von 3,94 (sog. Sugar Ratio).

2. Unter pathologischen Bedingungen:

   a) Akute Polyarthritis: Bei 5 Fällen mit akuter Polyarthritis ergaben sich durchwegs positive CRP-Reaktionen, obschon die Blutentnahme nicht in allen Fällen im akuten Stadium vorgenommen worden war, was an den

verschieden hohen Senkungswerten (4 - 47 mm in 1. Stunde) ohne weiteres zu erkennen ist. Auch die Diphenylaminquotienten wiesen hier eine markante Erhöhung auf (5,95 - 7,88).

b) Akuter Schub bei primär chronischer Polyarthritis: Ganz ähnliche Verhältnisse wie bei der akuten Polyarthritis zeigten 6 Fälle von primär chronischer Polyarthritis mit akutem Schub. Neben durchweg deutlich bis sehr stark positiven CRP-Reaktionen fanden sich hier erhöhte Senkungswerte von 20 - 100 mm in der 1. Stunde sowie hohe Diphenylaminquotienten (5,12 - 8,9).

c) Primär chronische Polyarthritis ohne Schub: Eine ganz andere Situation ergab sich in 22 Fällen von primär chronischer Polyarthritis ohne akuten Schub. Trotz gleichgrossen Senkungswerten wie bei der akuten Polyarthritis (5 - 45 mm in 1. Stunde) fiel hier die CRP-Reaktion stets negativ aus, während die Diphenylaminreaktion in 20 von 22 Fällen erhöhte Quotienten (5,0 - 11,7) aufwies.

d) Primär degenerative Gelenkerkrankungen (Arthrosen): Erwartungsgemäss liessen sich bei 5 Arthrosenfällen keine positiven CRP-Reaktionen beobachten. Die Senkungswerte schwankten zwischen 2 - 12 mm in der 1. Stunde, während die Diphenylaminreaktion hier allein mit stark pathologischen Werten imponierte (5,5 - 7,63).

e) Andere entzündliche und nicht entzündliche Erkrankungen: Bei nicht rheumatischen Erkrankungen kommen ebenfalls positive CRP-Reaktionen und stark pathologische Diphenylaminquotienten vor, womit die Unspezifität dieser Proben erwiesen ist.

3. Bewertung der CRP-Reaktion, der Diphenylaminreaktion und der Senkungsreaktion:

Es besteht keine Uebereinstimmung zwischen den Resultaten der drei genannten unspezifischen Reaktionen. Die CRP-Reaktion scheint auf ein akut entzündliches Geschehen hinzuweisen, während die Senkungsreaktion bekanntlich auch bei chronisch entzündlichen Prozessen und neoplastischen Vorgängen erhöht sein kann. Die Diphenylaminreaktion weist ein noch breiteres Reaktionsspektrum auf, indem selbst Fälle mit normaler Senkung erhöhte Diphenylaminquotienten zeigen.

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